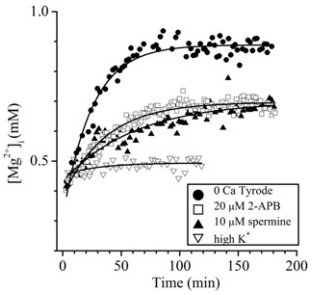
3372-Pos Board B233

Physiological Magnesium Influx Pathways in Cardiac Myocytes

Michiko Tashiro, Hana Inoue, Masato Konishi.
Dept. physiol. Tokyo medical Univ., Tokyo, Japan.

Rat ventricular myocytes were loaded with a fluorescent Mg^{2+} indicator furatpra (mag-fura-2), and intracellular free Mg^{2+} concentration ($[Mg^{2+}]_i$) was measured. Incubation of the cells in a Mg^{2+}-free Ca^{2+}-free high-K^+ solution for 20 min caused a decrease in $[Mg^{2+}]_i$ from ~0.9 mM to 0.2-0.5 mM (Mg^{2+} depletion). When the Mg^{2+}-depleted cells were then perfused with Ca^{2+}-free Tyrode's solution that contained normal levels of Na^+, K^+ and Mg^{2+}, $[Mg^{2+}]_i$ gradually returned back to the basal level. The time course of the $[Mg^{2+}]_i$ recovery was well fitted by a single exponential function with an average time constant of 42 min (25°C). We analyzed the first derivative of the fitted curve at time 0 (initial $d[Mg^{2+}]_i/dt$) as an initial rate of Mg^{2+} influx. The initial $d[Mg^{2+}]_i/dt$ was, on average, 0.27 ± 0.04 μM/s, and was unchanged by the presence of 1 mM extracellular Ca^{2+} (0.23 ± 0.03 μM/s). Membrane depolarization by high K^+ significantly decreased the rate to 0.053 ± 0.02 μM/s. The initial $d[Mg^{2+}]_i/dt$ was also significantly reduced by 20 μM 2-APB (0.13 ± 0.04 μM/s) or 10 μM spermine (0.14 ± 0.02 μM/s), known inhibitors of TRPM7/MIC channels. The results suggest that TRPM7/MIC channels serve as a physiological influx pathway for Mg^{2+} in cardiac myocytes.



3373-Pos Board B234

Mechanistic Insights into the Twin-Arginine Translocation Cycle of Escherichia Coli by an In Vivo Single-Molecule Approach

Felix Oswald[1], Siet M.J.L. van den Wildenberg[2], Kah Wai Yau[3], Peter van Ulsen[1], Gijs J.L. Wuite[1], Yves J.M. Bollen[1], Erwin J.G. Peterman[1].
[1]VU University Amsterdam, Amsterdam, Netherlands, [2]Leiden University, Leiden, Netherlands, [3]Utrecht University, Utrecht, Netherlands.

The twin-arginine translocation (Tat) system is a unique protein targeting pathway which has been found in bacteria, archea and chloroplasts as well as in plant mitochondria. Its ability to transport fully folded proteins across the cytoplasmic membrane distinguishes it from other translocation pathways. In E. coli the essential components of the system, TatA, TatB, and TatC, have been isolated in complexes of different size, suggesting that a fully active Tat complex forms only transiently. Furthermore, the precise steps in the translocation cycle remain mostly unknown.

We perform fluorescence microscopy and single-particle tracking to gain deeper insight into the dynamics of the Tat machinery. To this end, bacteria expressing low levels of GFP-fused Tat subunits are imaged with sensitive laser-illuminated wide-field fluorescence microscopy. Mobile fluorescent spots are observed, and their intensity and location determined by fitting them with a 2D Gaussian function. The trajectories of these spots are then established by linking the Gaussian fits in successive frames.

Our data shows that diffusion of TatA-eGFP is heterogeneous, and that its average diffusion coefficient decreases when translocation substrate is overexpressed. Moreover, TatA-eGFP mobility depends on the existence of an electrochemical potential, which is the driving force behind the Tat translocation system. This could suggest that TatA-eGFP complexes undergo a topological transition upon becoming 'translocation active'.

Membrane Receptors & Signal Transduction II

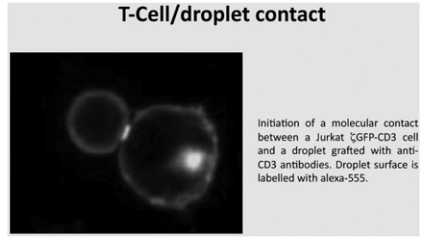
3374-Pos Board B235

Model Liquid Droplets to Explore T-Cell Surface Dynamics upon Activation

Nadia Bourouina[1], Claire Hivroz[2], **Nelly Henry**[3].
[1]Institut Curie/CNRS UMR 168, Paris, France, [2]Institut Curie/INSERM U932, Paris, France, [3]Institut Curie/CNRS UMR 168/ UPMC, Paris, France.

The T-cell is a central player of the immune response in mammals. It recognizes antigen on the surface of dedicated cells through the formation of several pairs of receptors and ligands in a micrometric bi-dimensional intercellular contact leading to cell activation. In order to simplify and dissect this process, we have developed model colloids grafted with defined ligands.

Here, we will show T-cell interaction with liquid colloids grafted with anti-CD3 targeted to the T-cell receptor (TCR) CD3ε subunit. Using fluorescence confocal microscopy, and ζCD3-GFP-expressing Jurkat cells, we have evidenced the active TCR-CD3 migration induced by anti-CD3 grafted droplets and the remarkable ligand dynamics induced by the cell contact on the droplet side. Cell activation was followed in parallel monitoring the intracellular calcium. Eventually, we have compared the dynamical properties of this cell-droplet contact with those of the contact driven by passive physico-chemical properties between two complementary droplets. Similarities between these two systems were limited to the initiation step of the process.



We concluded that the cell-droplet contact, conceived here as a simplified immune synapse, resulted from active cell mechanics and not from spontaneous self-assembly processes.

3375-Pos Board B236

The Molecular Basis for Lipid Recognition and Signaling in the Toll-Like Receptor Complex and a Family of Lipid-Binding Proteins

Teresa Paramo[1], Sally Higson[1], Thomas J. Piggot[2], **Peter J. Bond**[1].
[1]University of Cambridge, Cambridge, United Kingdom, [2]University of Southampton, Southampton, United Kingdom.

The transmembrane toll-like receptors (TLRs) are the initial gateway to almost all mammalian inflammatory responses to invading microbes. They are specialized for binding ligands with diverse structural and physiochemical properties ranging from microbial cell wall components to nucleic acids, and there is substantial interest in their pharmacological manipulation. TLR4 binds specifically to lipopolysaccharide (LPS), the central glycolipid component of Gram-negative bacterial outer membranes. Minute amounts of lipid A, the bioactive component of LPS, are an early sign of infection, and the association of TLR4 with many infectious and inflammatory diseases emphasizes its therapeutic importance. Subtle variations in the structure of lipid A profoundly affect activity, leading to unpredictable changes in TLR4 immune responses. To dissect the mechanisms of recognition and signaling, an extensive, explicitly-solvated, all-atom molecular dynamics simulation study has been performed for the isolated lipid-binding MD-2 co-receptor, and for the entire active, multimeric TLR4/MD-2 receptor complex, in the presence of a range of bound lipid A analogues, including several synthetic immunomodulatory mimetics undergoing clinical trials. We show that concerted large-scale conformational changes in MD-2 control the signaling status of the receptor complex, responding to variations in ligand acyl tail composition and headgroup phosphorylation status. Moreover, the co-receptor is part of a lipid-recognition superfamily, comprising immunoglobulin-like proteins involved in lipid signaling, transfer, and metabolism. Thus, we also report simulations of a wide range of these proteins, with or without ligands (including polyethers, fatty acids, single-chain lipids, and sterols), which reveal ligand-responsive conformational dynamics comparable to TLR4/MD-2. Therefore, a shared mechanism is identified in these distantly related family members, explaining variations in available structures obtained using different experimental approaches, and suggesting a molecular basis for allergic mimicry in dust-mite allergen lipid-binding proteins.

3376-Pos Board B237

A Common Model for Cytokine Receptor Activation: Combined Scissor-Like Rotation and Self-Rotation of Receptor Dimer Induced by Class I Cytokine

Xiaodong Pang, Huan-Xiang Zhou.
Department of Physics and Institute of Molecular Biophysics, Florida State University, Tallahassee, FL, USA.

The precise mechanism by which the binding of a class I cytokine to the extracellular domain of its corresponding receptor transmits a signal through the cell membrane remains unclear. Receptor activation involves a cytokine-receptor complex with a 1:2 stoichiometry. Previously we used our transient-complex theory to calculate the rate constant of the initial cytokine-receptor binding to form a 1:1 complex. Here we computed the binding pathway leading to the 1:2 activation complex. Three cytokine systems (growth hormone, erythropoietin, and prolactin) were studied, and the focus was on the binding of the extracellular domain of the second receptor molecule after forming the 1:1 complex. According to the transient-complex theory, translational and rotation diffusion of the binding entities bring them together to form a transient complex, which has near-native relative separation and orientation but not the short-range specific native interactions. Subsequently conformational rearrangement leads to the formation of the native complex. We found that the

changes in relative orientations between the two receptor molecules from the transient complex to the 1:2 native complex are similar for the three cytokine-receptor systems. We thus propose a common model for receptor activation by class I cytokines, involving combined scissor-like rotation and self-rotation of the two receptor molecules. Both types of rotations seem essential: the scissor-like rotation separates the intracellular domains of the two receptor molecules to make room for the associated Janus kinase molecules, while the self-rotation allows them to orient properly for transphosphorylation. This activation model explains a number of experimental observations. The transient-complex based approach presented here may provide a strategy for designing antagonists and be useful for elucidating activation mechanisms of other receptors.

3377-Pos Board B238

Diffusion of Single B Cell Receptors in Resting and Stimulated B Lymphocytes using Super-Resolution Microscopy

Matthew B. Stone, Marko Martinovski, Veatch L. Veatch.
University of Michigan, Ann Arbor, MI, USA.

The B cell antigen receptor (BCR) is an integral part of our immune systems that communicates binding of antigen in the extracellular environment through the plasma membrane to the cytoplasm. Antigen binding to the BCR results in phosphorylation of intracellular tyrosine activating motifs (ITAMs), which act as binding partners for SH2 domain containing adapters and Src family kinases. To examine the spatio-temporal dynamics of the BCR during stimulation, we utilize stochastic optical reconstruction microscopy (STORM) in live CH27 cells. Using Alexa-647 labeled anti-IgM Fab fragments, we resolve the BCR down to tens of nanometers with acquisition rates of 40 frames per second. We then examine BCR diffusion by tracking the localized receptors and found that the BCRs in resting cells exhibit a lognormal distribution of diffusion constants centered at 10-1 μm2/s. Upon stimulation, this population is quickly shifted to one centered at 10-2 μm2/s. In separate experiments, we examined the calcium response of CH27 B cells and found that the buffers used for STORM experiments do not alter calcium responses after receptor crosslinking. We also perform an auto correlation analysis of the localized receptors, which shows an increase in BCR clustering on the same timescale as the reduction in diffusion. In addition, we are investigating the dynamics of other proteins involved in BCR signaling, namely the Src family kinase Lyn and Ezrin. We use two color simultaneous emission of a photoswitchable fluorescent protein mEos2 in conjunction with Alexa-647 in fixed and live cells to resolve the behavior of these interaction partners with respect to the BCR.

3378-Pos Board B239

Rho Family Proteins in High- and Low-Affinity fMLF Receptors Signaling in Neutrophils

Julia Filina[1], Valentina Safronova[1], Aida Gabdoulkhakova[2].
[1]Institute of Cell Biophysics, Puschino, Russian Federation, [2]Kazan State Medical Academy, Kazan, Russian Federation.

Bacterial or mitochondrial peptide N-formyl-MLF (fMLF) binding with specific receptor activates chemotaxis, secretory degranulation and oxidative burst of the neutrophils. High and low affinity fMLF receptors (FPRs) can initiate different patterns of signaling components (Fu et al., 2004; Safronova, Gabdoulkhakova, 2009). Main functions of Rho-family proteins are signal transduction from various membrane receptors and regulation of the cytoskeleton. We suppose that small G-proteins are the node of FPRs signal transduction and divergence. Aim of the work was to find out the role of Rho family small G-proteins in signaling from high- and low-affinity receptors to NADPH oxidase. The study was carried out on the induced peritoneal neutrophils of Balb/c male mice. The immunofluorescent staining with specific Abs against RhoA and Rac1/2 was used to visualize the localization of Rho-proteins in the cells. Rho-proteins are evenly distributed in cytoplasm of non-stimulated cells. Translocation of Rho-GTPases to membrane occurs under the stimulation of neutrophils with fMLF that indicates their activation. High dose of fMLF (50 mkM) induces brighter fluorescence of juxtamembrane area in comparison with low dose (1 mkM). Formylated peptide fMLF stimulates dose-dependent activation of the neutrophil respiratory response. Rho activator reduces intensity of the respiratory burst under the stimulation of high-affinity receptors, but does not influence the respiratory burst initiated by low-affinity FPRs. Rac/Cdc42 activator also down-regulates the respiratory response to low concentration of fMLF without effect in case of high dose. Thus, Rho-family small GTPases participate in signal transduction from the FPRs and reduce activity of NADPH-oxidase.in the stimulation of the high-affinity FPRs. This effect of Rho-family proteins can endow the reciprocal relationships between chemotaxis and oxidative activity in process of the neutrophil movement within the gradient of the chemoattractants.

3379-Pos Board B240

Activation of the Innate Immune Receptor Dectin-1 by Clustering

Amira Fitieh[1], Andrew Locke[1], Tomek Lipinski[2], Sandra Ungarian[1], David Bundle[2], Khuloud Jaqaman[3], **Nicolas Touret**[1].
[1]Dpt of Biochemistry, University of Alberta, Edmonton, AB, Canada, [2]Dpt of Chemistry, University of Alberta, Edmonton, AB, Canada, [3]Dpt of Systems Biology, Harvard Medical School, Boston, MA, USA.

Dectin-1 is a pattern-recognition receptor that plays a key role in anti-fungal immunity. It specifically recognizes beta-glucans, polymers of glucose found on the fungal cell wall. Upon ligand binding, dectin-1 triggers signaling cascades that lead to various cellular responses, including phagocytosis and the release of pro-inflammatory mediators, which all culminate in the generation of an effective immune response and eradication of the pathogen. The molecular mechanisms underlying dectin-1 activation and signal transduction are not fully understood. The aim of this study is to elucidate such mechanisms. We propose that upon binding of fungal particles, dectin-1 receptors cluster and assemble into multimeric complexes. In these clusters, dectin-1 becomes activated and promotes the recruitment and activation of Syk (spleen tyrosine kinase), which in turn triggers subsequent signaling cascades. To test our hypothesis, we stably expressed human dectin-1 in RAW 264.7 macrophages. Various levels of dectin-1 clustering were induced using antibody cross-linking or beta-glucans of different molecular weights. The corresponding stimulation of dectin-1 was detected by determining the activation of several downstream effectors (Src, Syk, NF-kB). We demonstrate that antibody cross-linking and larger ligands are able to induce more Syk phosphorylation than smaller ligands. To quantify the level of dectin-1 clustering, single molecule analysis was employed. Upon antibody cross-linking or ligand binding dectin-1 formed nanodomains of about 500 nm of diameter, which were identified as the nucleation site for intracellular signaling. Additionally, the phosphorylation and recruitment of Syk to regions of the plasma membrane rich in dectin-1 clusters was observed using confocal microscopy. Together, our results suggest that receptor clustering, is the mechanism by which dectin-1 is activated.

3380-Pos Board B241

Signal Processing by the Control Cycle of the IKK Kinase in the NFκB Signaling Axis

Marcelo Behar, Alexander Hoffmann.
UC San Diego, La Jolla, CA, USA.

The inflammatory response transcription factor NFkB, a key component of the immune system, shows intricate stimulus-specific temporal dynamics. It has been proposed that those dynamics play a role controlling the genetic outcome of inflammatory signals and hence the specificity of the cellular response to cytokines and other stimuli. As malfunctions in NFkB signaling are linked to many immune diseases as well as the onset and development of cancers, it is of considerable clinical interest to understand the mechanisms that control NFkB dynamics. Here we focus on IKK, a hub kinase that targets the main negative regulators of NFκB activity and onto which diverse receptor associated signals converge. Biochemical evidence suggests that IKK is regulated via a multi-state regulatory cycle and therefore we hypothesize that it can operate as a modulator, actively reshaping the signals generated at the receptor proximal level. Here we demonstrate that the IKK control cycle can function in at least three dynamical regimes some of them producing signals comprising multiple temporal phases with distinct coding capabilities. In particular, we show that the simplest three-state regulatory cycle generates biphasic signals with an early phase well suited for relaying information about stimuli amplitude and a late phase more apt for encoding stimulus duration. This study demonstrates that an actively regulated hub kinase can play a crucial role functioning as a signal ‘‘categorizer’’ classifying complex incoming signals into a limited set of output activities. Expanding the general analysis to a more detailed model of IKK regulation revealed how specific features of IKK and NFkB activities are controlled by the different enzymatic mechanisms within the kinase cycle.

3381-Pos Board B242

Integrating Single Molecule Techniques to Investigate Antigen-Independent Effects of IgE Binding to FcεRI

Samantha L. Schwartz[1], Qi Yan[2], Patrick J. Cutler[1], Marcel P. Bruchez[2], Keith A. Lidke[1], Diane S. Lidke[1].
[1]University of New Mexico, Albuquerque, NM, USA, [2]Carnegie Mellon University, Pittsburgh, PA, USA.

Using single particle tracking and super-resolution, we have examined the influence of IgE binding on FcεRI mobility and distribution. The high affinity IgE receptor, FcεRI, of mast cells is an abγ2 tetramer, where the a subunit binds IgE and the b and γ subunits contribute ITAM-motifs for signaling. IgE binding is known to stabilize the receptor on the cell surface and recent evidence has